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Why is mucormycosis more difficult to cure than more common mycoses?

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Abstract

Although considered to be a rare infection, mucormycosis (zygomycosis) has emerged as the second most common invasive mould infection. Despite the advent of newer antifungal agents, mortality rate of mucormycosis remains exceedingly high. Successful management of mucormycosis requires early diagnosis, reversal of underlying predisposing risk factors, surgical debridement and prompt administration of active antifungal agents. However, mucormycosis is not always amenable to cure. There are challenging obstacles that lead to difficulties in management of amphotericin B. These include unique host-based risk factors for mucormycosis, the fungus' resistance to innate host defences and distinctive features of its immunopathogenesis, such as extensive angioinvasion, increased virulence and use of chelators by the fungus as siderophores. In addition to these obstacles, the difficulties in early diagnosis, including nonspecific clinical manifestations, lack of serological methods, as well limitations of culture and molecular methods, lead to delay in initiation of antifungal therapy. Finally, the variability of susceptibility to amphotericin B and resistance to most other conventional antifungal agents leads to major limitations in successful treatment of this devastating infection.

Keywords: Amphotericin B, *Aspergillus fumigatus*, mucormycosis, *Rhizopus oryzae*, zygomycosis
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Introduction

Invasive fungal infections are important causes of morbidity and mortality especially in immunocompromised patients. While *Candida* spp., *Aspergillus* spp. and *Cryptococcus neoformans* are responsible for the preponderance of invasive fungal infections, other less common fungal pathogens account for a significant proportion of morbidity and mortality [1]. Among these pathogens with worldwide distribution and increasing frequency are Zygomycetes causing mucormycosis (previously called zygomycosis) [2]. Mucormycosis is caused by fungi of the order Mucorales and the family Mucoraceae [3]. *Rhizopus*, *Mucor*, and *Lichtheimia* (formerly *Absidia*) are the most common genera that cause mucormycosis, accounting for 70–80% of all cases, whereas *Cunninghamella*, *Apophysomyces*, *Saksenaea*, *Rhizomucor*, *Cokeromyces*, *Actinomucor* and *Syncephalastrum* are responsible for <1–5% of reported cases [4].

A feature that is characteristic for Mucorales among other filamentous fungi is that they cause disproportionately serious infections with exceedingly high morbidity and mortality in relatively immunocompetent individuals [5]. This may be particularly observed in patients with diabetes mellitus, neonates, as well as those with wounds caused by surgery, trauma and burns. Despite aggressive surgical intervention and intensive antifungal treatment, mortality of mucormycosis is high, ranging from 50–100% depending on the disease form [3,5,6]. By comparison, mortality rates for candidiasis and aspergillosis range from 20–50% and 35–45%, respectively [7–9].

Successful management of mucormycosis requires early diagnosis, reversal of underlying predisposing risk factors,

surgical debridement and prompt administration of amphotericin B [10]. However, despite these measures, mucormycosis is not always amenable to cure. We will discuss, in this article, the challenging obstacles that lead to difficulties in management of mucormycosis (Table 1).

Patient Population at Risk for Mucormycosis

Mucormycosis is an infection affecting all ages, from premature neonates to elderly people with varying types of underlying conditions [5,11–13]. While the incidence rates of invasive mucormycosis in these diverse patient groups have not been well studied, the current data demonstrate increased incidence in a wide range of specific immunocompromised patient groups [2,5,11].

In the most contemporary epidemiologic study of mucormycosis in Europe, the most common underlying diseases were haematological malignancies (44%) followed by diabetes mellitus (17%) and trauma (17%) [2]. Data from a global fungal infection registry that included patients from central Europe and Asia demonstrated that malignancy (63%), diabetes (17%) and solid organ transplantation (10%) predominated as underlying conditions for mucormycosis [11].

A comprehensive review of 929 reported cases showed that the most common underlying condition were diabetes (36%), followed by malignancy (17%), solid organ transplantation (7%), deferoxamine therapy (6%), injection drug use (5%) and bone marrow transplantation (5%). In addition, the patients' underlying conditions were shown to be related with the pattern of infection, in that, patients with malignancy had more likely pulmonary infection; whereas, patients with diabetes mellitus had sino-orbital and rhinocerebral disease [5,14]. This relationship would be, in all probability, a reflection of the host's immune function along with other pathological-anatomical factors [5].

However, it has also been postulated that the differences in disease pattern may reflect the diversity between developed and developing countries from where the cases were reported [15]. Specifically, in developed countries, most patients with mucormycosis are those with diabetes and haematological malignancies [6], while in developing countries those with uncontrolled diabetes and trauma predominate [16,17]. Notably, there is a relatively large proportion (*c.* 14% in children and 19% in adults) with no apparent immunocompromising conditions at the time of infection [5,18]. Within France, traumatic wounds in immunocompetent and immunocompromised patients comprise a distinct population [14].

The differences in the population at risk for mucormycosis depicted by the current studies could be explained by the period effect, publication bias or the contribution of different investigational centres in each study. However, it is characteristic that while other filamentous fungi affect more classically defined immunocompromised hosts, such as those with malignancies under chemotherapy, transplant recipients or inherited immunodeficiencies, mucormycosis can cause serious infections in relatively immunocompetent hosts, such as those with diabetes mellitus, on deferoxamine therapy, injection drug users and with no apparent immune defect other than a traumatic injury [5,18]. Correspondingly, taking also into account the ubiquitous nature of Mucorales, defining a specific population at risk for mucormycosis in order to target it is a challenging problem as 'candidate-patients' represent a quite heterogeneous group.

TABLE 1. Possible reasons for difficulty to treat mucormycosis

Variable underlying conditions of mucormycosis
Haematological malignancies
Diabetes
Solid organ transplantation
Haematopoietic stem cell transplantation
Injection drug use
Neonates
Trauma
Burns
Normal host
Deferoxamine therapy
Distinctive features of immunopathogenesis
Extensive angioinvasion
Increased virulence (exerted by some species, see text)
Use of chelators by fungus as siderophores
Difficulties in diagnosis
Non-specific clinical manifestations
Lack of validated or standardized serological methods
Difficulties in culturing organism
Limited availability of validated and standardized molecular methods
Limitations of antifungal therapy
Late or no diagnosis antemortem
Elevated MICs of amphotericin B
Higher MICs of *Cunninghamella* to amphotericin B
Triazole-specific variability of susceptibility
Species-specific variability of susceptibility to triazoles

Distinctive Features of the Immunopathogenesis of Mucormycosis

Medically important members of the order Mucorales share many features with other filamentous fungi such as portals of the host for infection (airways and disrupted mucocutaneous barriers), the main lines of innate host defences (phagocytes, specific ligands in fungal spores such as pathogen-associated molecular patterns (PAMPs) and immune cells such as Toll-like receptors (TLRs)), as well as histopathological and clinical features [19,20]. However, *Rhizopus oryzae* and other selected Mucorales possess unique virulence characteristics and exert distinctive host–pathogen interactions compared to other fungi facilitating, thus, host evasion and disease progression [21].

There are several lines of *in vitro* evidence showing that *R. oryzae* and other members of the Mucorales have reduced susceptibility to innate host defence as compared to other more common fungi, such as *Aspergillus fumigatus* or *Candida albicans* [22,23]. Moreover, differential interspecies susceptibility patterns to host responses exist within the order Mucorales [24–26]. Namely, members of the genus *Rhizopus* suffer less hyphal damage and stimulate an impaired oxidative burst in human phagocytes as compared to *Lichtheimia (Absidia)* spp. [24], and *Cunninghamella bertholletiae* shows, *in vitro,* increased resistance to phagocyte-induced hyphal damage and, *in vivo,* increased virulence in an experimental neutropenic pulmonary mucormycosis model in comparison with *Rhizopus* spp. [25,26]. In agreement are the results of the *Drosophila melanogaster* host model that simulates important aspects of mucormycosis in humans. In contrast to other fungi, species within the order Mucorales rapidly infect and kill *D. melanogaster* wild-flies, and their pathogenicity is linked with impaired phagocytic cell activity and hyphal damage compared with those of *A. fumigatus* [27]. These experimental findings are collectively consistent with epidemiological data and clinical experience showing greater prevalence of *Rhizopus* spp. compared to *Lichtheimia corymbifera* in immunocompromised patients [3,28–30] and increased mortality in patients with *C. bertholletiae* infection [4,5].

While the exact mechanisms underlying such variable responses against Zygomycetes have not yet been elucidated, the increased virulence exerted by some species has been associated with the induction of a more pronounced proinflammatory response by selected species [25,31]. It was postulated that differences in cell-wall constituents and ligands may lead to variable recognition of fungal cell wall recognition patterns by TLR and dectin receptors with consequent downstream altered expression of certain stimulatory molecules like chemokines and cytokines [24,31]. Indeed, the *D. melanogaster* model demonstrated the importance of fungal recognition for infection development showing that Toll-deficient flies exhibit increased susceptibility to infections caused by the Mucorales [27]. Whole-genome expression profiling in wild-type flies after infection with Mucorales vs. *A. fumigatus* revealed that genes acting on pathogen recognition, immune defence, stress response, detoxification, steroid metabolism or tissue repair are differentially regulated by these two fungal pathogens [27].

One of the critical characteristics in mucormycosis pathogenicity is the extensive angioinvasion that results in vessel thrombosis and tissue necrosis [6,32]. This angioinvasion leads to haematogenous dissemination of the organism to target organs, while ischaemic necrosis of the infected tissue can prevent leucocyte and antifungal agent penetration to the foci of infection [21]. *R. oryzae* was used as a model system in understanding the basis of fungal pathogenicity. Sequencing the genome of a pathogenic *R. oryzae* strain there was evidence that the entire genome had been duplicated and retained two copies of three extremely sophisticated systems involved in energy generation and utilization. This gene duplication has led to the development of gene families related to fungal virulence, fungal cell wall synthesis enzymes and signal transduction, which may contribute to the invasive nature of *R. oryzae* [33].

The seminal clinical observations that patients with diabetic ketoacidosis as well as patients receiving dialysis who are treated with iron chelator deferoxamine are characteristically susceptible to mucormycosis highlights the central role of host iron in the pathogenesis of mucormycosis [21,34]. In proof of principle *in vitro* studies, it was shown that *Rhizopus* spp. can accumulate 8- and 40-fold greater amounts of iron supplied by deferoxamine than can *A. fumigatus* and *C. albicans,* respectively [35]. Similarly, data from animal models showed that administration of deferoxamine or free ions reduced survival of animals infected with *Rhizopus* spp. but not *C. albicans* [36–38]. Deferoxamine *per se* is not the pathogenetic factor for infection, but *Rhizopus* spp. utilize deferoxamine as a siderophore to supply previously unavailable iron to the fungus [6]. However, not all Zygomycetes have the same susceptibility to iron chelators [39].

Among the classic enhancers of mucormycosis pathogenicity in humans is corticosteroid immunosuppressive therapy and diabetes mellitus, which seem to impair the ability of macrophages to prevent germination of sporangiospores [40,41]. Neutropenia encountered in patients with haematological malignancies and in the pre-engraftment stage of HSCT is a major contribution to the pathogenesis of mucormycosis [20,21,23–26]. Further contributing to the pathogenesis of mucormycosis is the presence of a receptor–ligand interaction between endothelial cell surfaces and *Rhizopus oryzae* [21]. More recently, it was found that exposure of Mucorales to voriconazole selectively enhanced their virulence [42]. Up to date, a number of potential virulence factors including mycotoxins and lytic enzymes excreted by *Rhizopus* spp. have been proposed; however, their contribution to Zygomycetes pathogenicity needs to be further elucidated [21].

Difficulties in Mucormycosis Diagnosis

Early and accurate diagnosis is, *a priori,* the most critical aspect for improved outcome of mucormycosis given the limited therapeutic options available, which frequently involve disfiguring and debilitating surgeries. However, many suspected mucormycosis cases ranging from 4 to >90% are not

confirmed until post-mortem examination [2,43–45]. The establishment of a definite diagnosis is hampered by a variety of factors including non-specific clinical presentation of mucormycosis as well as the various drawbacks of the currently implemented diagnostic means.

There are some clinical manifestations such as diplopia, necrotic naso-sinus eschars, pleuritic pain, necrotic cutaneous lesions that, in the predisposed host, carry a potentially high predictive value [15,43]. These clinical manifestations are discussed in greater depth elsewhere. Nevertheless, these manifestations are nonspecific and their differential diagnosis includes a range of infections caused by angioinvasive pathogens, including *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp. and *Pseudomonas aeruginosa*; therefore, a high index of suspicion and prompt identification of host predisposing factors are required for early recognition of mucormycosis.

Cultural isolation and identification of the fungus to the genus or species level is of epidemiological, prognostic and therapeutic importance [4,26,43]. The cultural isolation yield ranges from 50 to 71%, while it was documented that it was significantly improved over time [2,5]. However, recovery of Mucorales from specimens in clinical microbiology laboratories is challenging. Performing invasive procedures to obtain the necessary material especially in severely ill patients with thrombocytopenia or coagulopathy may be a daunting and perilous task. The hyphae of Mucorales may be difficult to observe on wet mounts and need special chitin-binding stains with a fluorescent microscope or may not be abundant so as to be seen. Moreover, tissue handling aggressive tissue grinding or homogenization may destroy the coenocytic hyphae [43,46]. While Mucorales are usually morphologically distinctive from other filamentous fungi, in some cases where antifungal therapy has commenced before biopsy, morphological features may be atypical, reducing the ability to differentiate Mucorales from other filamentous fungi [43].

Currently, there are no readily available nonculture methods, such as measurement of biochemical or serological biomarkers, to facilitate the diagnosis of invasive mucormycosis. By comparison, circulating mannan antigen and (1→3)-β-D-glucan are used for diagnosis of invasive candidiasis, while galactomannan measured in serum and bronchoalveolar lavage (BAL) fluid is useful in the diagnosis of invasive aspergillosis.

Molecular identification is feasible and holds promise for early and accurate diagnosis of mucormycosis. Most studies have evaluated ribosomal targets (18S, 28S and internal transcribed spacer (ITS)) or other DNA targets (the high-affinity iron permease 1 gene FTR1 or cytochrome b) that allow species identification of Zygomycetes from cultures [47]. Molecular methods also play a role in formalin-fixed, paraffin-embedded biopsy samples, which, on many occasions, constitute the only material available for use for diagnosis in the clinical setting.

The greater challenge for early diagnosis of mucormycosis is to develop molecular systems for identification of amplicon from blood or BAL fluid in patients with invasive pulmonary mucormycosis. One of the first laboratory animal studies to demonstrate circulating species-specific amplicon from plasma and BAL fluid in experimental invasive pulmonary mucormycosis found sensitivities of 67% and 100% with a high degree of specificity [48]. Other studies from experimental animal models as well as case reports from patients further demonstrate the feasibility in identifying Mucorales such as *C. bertholletiae, Rhizomucor pusillus, R. microsporus, L. corymbifera* and *Saksenaea vasiformis* from tissue and cultures [48–55]. In these cases molecular approaches are mainly based on PCR assays; however, the performance of these techniques can become compromised, as formalin fixation is associated with DNA damage. Currently, molecular diagnostics for mucormycosis is not widely available; its use is compromised by limited sensitivity, time to detection and ability to provide rapid results. Collectively, while molecular diagnosis of mucormycosis is feasible, several areas, such as DNA extraction methodology, identification of informative DNA targets, a validated sequence database, a broader spectrum of primers, as well as systematic validation and standardization of assays in human specimens warrant improvement [43,56].

A protocol of the European Confederation of Medical Mycology (ECMM)—International Society for Human and Animal Mycology (ISHAM) Zygomycosis Working Group will be systematically addressing these needs for advancing the development of antigenic, biochemical and molecular assays for diagnosis of invasive pulmonary mucormycosis. The three objectives of development of a predictive risk model, an archive of human specimens and an educational effort are aimed at advancing the early diagnosis of invasive mucormycosis.

Diagnostic imaging and especially CT scan is an invaluable tool to aid in early diagnosis of invasive infections in immunocompromised hosts. Nevertheless, radiological features are not pathognomonic of mucormycosis while their absence cannot exclude this infection [57]. Many lesions, such as nodules, halo sign, reverse halo sign, cavities, wedge-shaped and pleural effusions, characteristically occur in pulmonary mucormycosis as well as other angioinvasive organisms like *Aspergillus* spp., *Scedosporium* spp., *Fusarium* spp. and *P. aeruginosa* [43,57].

Early and accurate diagnosis of mucormycosis is an area of critical importance and until newer molecular diagnostic techniques and biomarkers become available, diagnosis will

be based on prompt recognition of risk factors, clinical manifestations and radiological findings and with conformation by culture and biopsy [43].

Difficulties in Treatment of Mucormycosis

The advent of newer antifungal agents significantly improved the prognosis in immunocompromised patients with invasive fungal infections. However, in the case of mucormycosis, the invasive nature of the disease leads to an overall mortality exceeding 50%, while on many occasions, antifungal therapy alone is rarely effective resulting in 100% mortality particularly for patients with disseminated disease [2,5,14] (Fig. 1).

Various Mucorales organisms have differential responses to antifungal agents. For example, *R. oryzae* tends to exhibit *in vitro* resistance to posaconazole; *Mucor circinelloides* shows greater susceptibility to posaconazole, and *Cunninghamella* tends to have higher MICs to amphotericin B [43]. In addition, the same species have variable response to the same antifungal class, for example *R. oryzae* has a variable response to different triazoles, including lack of activity of voriconazole as compared to relatively good activity of posaconazole. The recent sequencing of *R. oryzae* revealed that this strain is genetically equipped for adaptation to hostile environments such as the effects of antifungal agents. It has been advocated that the variable responses of *R. oryzae* to voriconazole, itraconazole and posaconazole are possibly due to the increased copy number and divergence of duplicated *ERG11* (the principal gene target for the triazoles) [33]. Consequently, the MICs for itraconazole and posaconazole are 4–8 dilutions higher for *R. oryzae* than those for *A. fumigatus,* and fungicidal activity is not achieved over a range of safely achievable drug concentrations [58–60]. The frequent breakthrough *R. oryzae* infections occurring in patients receiving voriconazole indicate that this antifungal agent is ineffective against *R. oryzae* [61,62]. This adaptive ability of *R. oryzae* may result in higher potential for development of resistance during long-term triazole therapy than *A. fumigatus* [63].

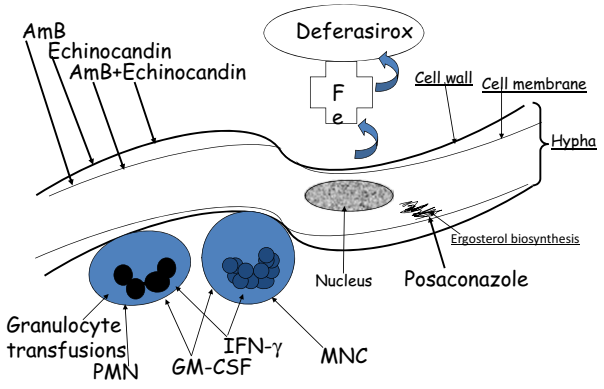

FIG. 1. Treatment modalities against mucormycosis. PMN, polymorphonuclear neutrophils; MNC, monocytes; GM-CSF, Granulocyte-Macrophage-Colony-Stimulating Factor; IFN-γ, Interferon-γ.

Amphotericin B is considered the first line therapy for mucormycosis. However, there are many lingering use problems to resolve, including the optimal dosage and timing for treatment initiation for each site of infection. Many clinicians who treat mucormycosis in an effort to control the infection use maximum tolerance doses of liposomal amphotericin B with a risk for nephrotoxicity. Studies from animal models showed that higher amphotericin B tissue concentration may be required for effective treatment of mucormycosis as compared to aspergillosis [64–66], while a phase II clinical trial of high-dose liposomal amphotericin B (10 mg/kg/day, IV) in mucormycosis treatment has been recently completed pending the efficacy results (http://clinicaltrials.gov/show/NCT00467883).

The impact of prompt amphotericin B-based treatment on the outcome of patients with mucormycosis has been determined by a retrospective study [67]. Chamilos *et al.* [67] demonstrated that delayed amphotericin B-based therapy resulted in a two-fold increase in mortality in patients with haematological malignancy and mucormycosis compared with early treatment (83% vs. 49%). Furthermore, delayed treatment of invasive mucormycosis was an independent predictor of poor outcome in multivariate analysis (odds ratio, 8 (95% confidence interval, 1.7–38.2); p 0.008). Given that Mucorales grow rapidly *in vivo*, there is a 'window of opportunity', which is much shorter than that of aspergillosis, where effective treatment should be initiated before extensive angioinvasion and dissemination occur [63]. Notably, while *in vitro* echinocandins demonstrate virtually no activity against Mucorales, *in vivo,* they are modestly effective [63]. Notably, at higher doses, echinocandins have attenuated activity compared with lower doses possibly reflecting upregulation of homeostatic cell-wall responses in the fungi that 'rescue' the fungus from the effects of echinocandins through compensatory increase in chitin synthesis [68,69]. In this regard, treatment options with predictable and favourable pharmacokinetics, such as lipid formulations of amphotericin B with echinocandin provide a reasonable rationale for phase III randomized, double-blinded, placebo-controlled trials [63]. However, more work on the safety, tolerability, efficacy, pharmacokinetics and pharmacodynamics is needed in a wider range of animal models of invasive pulmonary mucormycosis to assure that this hypothesis is tenable before embarking on a phase III trial.

Conclusions

Mucormycosis has a worse outcome than other invasive fungal infections such as candidiasis or aspergillosis. The higher degree of difficulty to cure this devastating infection is related to differences in host–fungus interactions, and pathogenetic mechanisms, as well as greater difficulties in early diagnosis when the 'window' of successful treatment is higher, and wider inadequacies of therapeutic options. Further advances in understanding host defence, developing newer diagnostic tools and creating better therapeutic interventions may improve outcome of this devastating disease.

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